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(54) **REMEDIES FOR CANCER**

(57) It is provided that type-X $sPLA_2$ inhibitors are useful in preventing or treating cancer.

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Description

Technical Field

[0001] The present invention relates to a composition for the prevention or treatment of cancer which contains an inhibitor against type-X $sPLA_2$ (secretary PLA_2) as an active ingredient.

Background Art

[0002] It is described in Cell. (1996) 87 803-809 that inhibitors of COX-2 at a downstream position of PLA_2 in arachidonate cascade are useful for the treatment of rectosigmoid cancer. As the method of diagnosing tumor including prostate cancer, a quantitative and qualitative assay to detect $sPLA_2$ proteins or $sPLA_2$ mRNA levels is described in WO98/05349 (JP Laid-Open (Tokukai) No. 2001/500847). However, neither of the documents describes that compounds having inhibitory activities against type-X $sPLA_2$ are effective for the treatment of cancer.

Disclosure of Invention

[0003] The inventors of the present invention examined the expression of type-X $sPLA_2$ in various kinds of human pathological tissues with anti-type-X $sPLA_2$ antibody. They found the elevated expression of type-X $sPLA_2$ in several tumor tissues.

[0004] The immunohistochemical analysis of tumor tissues was performed as follows. At first, anti-human type-X $sPLA_2$ antibody was added to the slides prepared from normal adult tissues or tumor tissues from cancer patients and incubated for several hours. Next, in order to examine the expression of type-X $sPLA_2$ in the tissues, the expression of type-X $sPLA_2$ was visualized by using the methods such as the immunohistochemical labeling to detect the type-X $sPLA_2$ signals. Consequently, the type-X $sPLA_2$ signals were detected in the slides prepared from tumor tissues, suggesting that the expression of type-X $sPLA_2$ is elevated in tumor tissues.

[0005] In addition, the inventors of the present invention performed the experiments for neutralization of type-X $sPLA_2$ signals. Precisely, before the addition of anti-human type-X $sPLA_2$ antibody to the slides, the slides were incubated with the purified type-X $sPLA_2$ protein for several hours. Hereafter, the slides were processed as the same procedures as described above to examine the type-X $sPLA_2$ signals. Consequently, the type-X $sPLA_2$ signals were disappeared in the slides prepared from tumor tissues.

[0006] Thus, the elevated expression of type-X $sPLA_2$ was confirmed in human tumor tissues.

[0007] Furthermore, the inventors of the present invention examined the inhibitory effects of $sPLA_2$ inhibitor against the type-X $sPLA_2$-induced release of oleic acid from tumor cell. Consequently, they confirmed that $sPLA_2$ inhibitors significantly blocked the type-X $sPLA_2$-induced release of oleic acid from tumor cells.

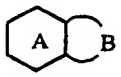
[0008] On the other hand, potential involvement of PGE_2 in the development of tumors has been described in Cancer Research 59, 5093-5096, 1999. Then, the inventors of the present invention examined the inhibitory effects of $sPLA_2$ inhibitors against the type-X $sPLA_2$-induced PGE_2 production in tumor cells. Consequently, they confirmed that $sPLA_2$ inhibitors significantly blocked the type-X $sPLA_2$-induced PGE_2 production in tumor cells.

[0009] The inventors of the present invention achieved the following present invention based on the above experimental results.

[0010] That is to say, the present invention relates to I) a composition for prevention or treatment of cancer which contains a type-X $sPLA_2$ inhibitor as an active ingredient.

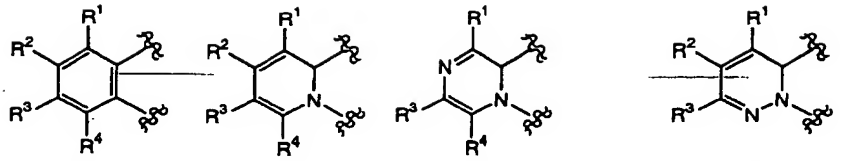
[0011] In more detail, the present invention relates to the following II) to XV).

II) A composition for prevention or treatment of cancer which contains as an active ingredient a compound represented by the formula (I):



(I)

wherein Ring A is represented by the formula (a) to (d):

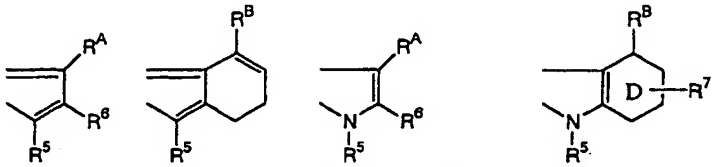


(a) , (b) , (c) , or (d)

wherein R^1 and R^2 are each independently hydrogen atom, non-interfering substituent, or -(L^1)-(acidic group) wherein L^1 is an acid linker having an acid linker length of 1 to 5, provided that one of the R^1 and R^2 is -(L^1)-(acidic group);

R^3 and R^4 are each independently hydrogen atom, non-interfering substituent, carbocyclic group, carbocyclic group substituted with a non-interfering substituent(s), heterocyclic group, or heterocyclic group substituted by a non-interfering substituent(s); and

-B- is represented by the formula (e) to (h):



(e) , (f) , (g) , or (h)

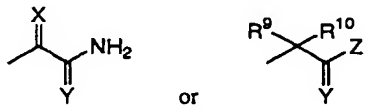
wherein R^5 is (j) C1 to C20 alkyl, C2 to C20 alkenyl, C2 to C20 alkynyl, carbocyclic group, or heterocyclic group, (k) the group represented by (j) each substituted independently with at least one group selected from non-interfering substituents, or -(L^2)-R^8 wherein L^2 is a divalent linking group of 1 to 18 atom(s) selected from hydrogen atom(s), nitrogen atom(s), carbon atom(s), oxygen atom(s), and sulfur atom(s), and

R^8 is a group selected from the groups (j) and (k);

R^6 is hydrogen atom, halogen, C1 to C3 alkyl, C3 to C4 cycloalkyl, C3 to C4 cycloalkenyl, C1 to C3 alkyloxy, or C1 to C3 alkylthio;

R^7 is hydrogen atom or non-interfering substituent;

R^A is represented by the formula:



or

wherein R^9 and R^{10} are each independently hydrogen atom, C1 to C3 alkyl, or halogen;

X and Y are each independently oxygen atom or sulfur atom; and

Z is $-NH_2$ or $-NHNH_2$;

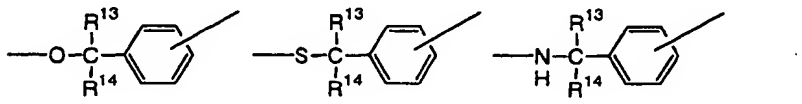
R^B is $-CONH_2$ or $-CONHNH_2$; and,

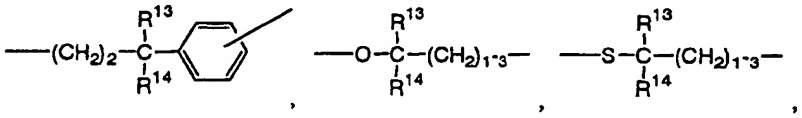
Ring D is cyclohexene ring or benzene ring;

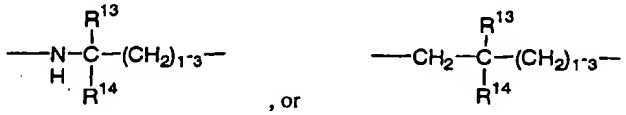
provided that Ring A is (b), (c), or (d) when -B- is (e) or (f), a prodrug thereof, its pharmaceutically acceptable salt, or its solvate.

III) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in II) as an active ingredient, wherein R^1 is hydrogen atom or $-(L^3)-R^{11}$ wherein L^3 is $-OCH_2-$, $-SCH_2-$, $-NH-CH_2-$, $-CH_2-CH_2-$, $-O-CH(CH_3)-$, or $-O-CH(CH_2CH_2C_6H_5)-$; R^{11} is $-COOH$, $-CONHSO_2C_6H_5$, $-SO_3H$, or $-P(O)(OH)_2$; and

R^2 is hydrogen atom or $-(L^4)-R^{12}$ wherein L^4 is represented by the formula:



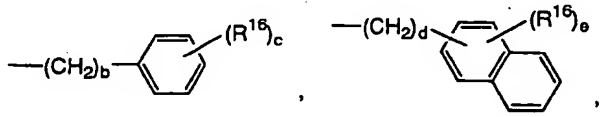


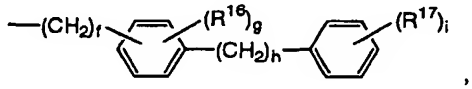


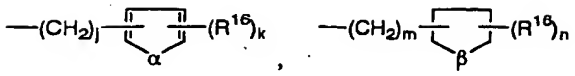
wherein R^{13} and R^{14} are each independently hydrogen atom, C1 to C10 alkyl, C1 to C10 aralkyl, carboxy, alkyloxycarbonyl, or halogen; R^{12} is $-COOH$, $-SO_3H$, or $-P(O)(OH)_2$, provided R^1 and R^2 are not hydrogen atom at the same time.

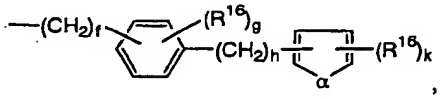
IV) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in II) or III) as an active ingredient, wherein R^3 is hydrogen atom, C1 to C6 alkyl, C3 to C6 cycloalkyl, aryl, or a heterocyclic group and R^4 is hydrogen atom or halogen.

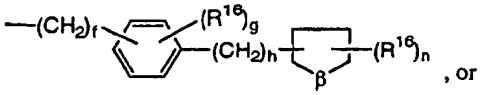
V) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in any one of II) to IV) as an active ingredient, wherein R^5 is $-(CH_2)_{1-6}-R^{15}$ wherein R^{15} is represented by the formula:

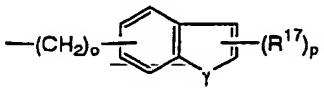






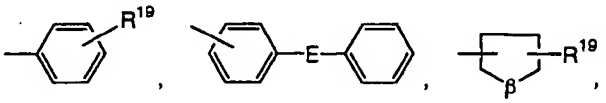


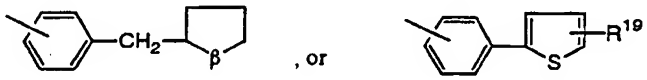




wherein b, d, f, h, j, m, and o are independently an integer from 0 to 2; R^{16} and R^{17} are each independently halogen, C1 to C10 alkyl, C1 to C10 alkyloxy, C1 to C10 alkylthio, aryloxy, or C1 to C10 haloalkyl; α is oxygen atom or sulfur atom; β is $-CH_2-$ or $-(CH_2)_2-$; γ is oxygen atom or sulfur atom; c, i, and p are independently an integer from 0 to 5; e is an integer from 0 to 7; g is an integer from 0 to 4; k and n are each independently an integer from 0 to 3.

VI) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in V) as an active ingredient, wherein R^5 is $-CH_2-R^{18}$ wherein R^{18} is represented by the formula:





wherein β is $-CH_2-$ or $-(CH_2)_2-$; R^{19} is hydrogen atom, C1 to C3 alkyl, or halogen; E is a bond, $-CH_2-$ or -O-.

VII) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in any one of II) to VI) as an active ingredient, wherein R^1 is $-OCH_2COOH$.

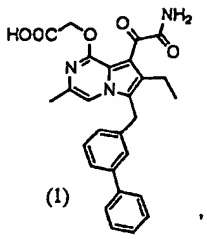
VIII) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in any one of II) to VII) as an active ingredient, wherein R^2 is hydrogen atom.

IX) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in any one of II) to VIII) as an active ingredient, wherein R^6 is C1 to C3 alkyl.

X) A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as described in any one of II) to IX)as an active ingredient, wherein R^A

is $-CH_2CONH_2$ or $-COCONH_2$.

XI) A composition for prevention or treatment of cancer which contains a compound as an active ingredient represented by the formula: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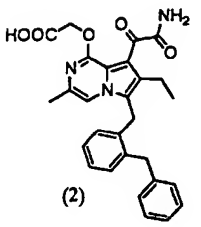
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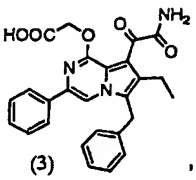
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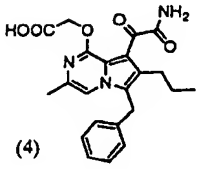
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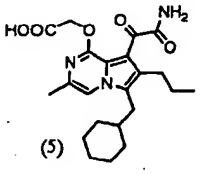
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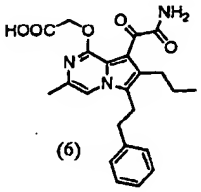
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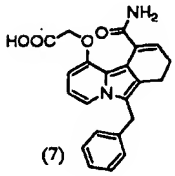
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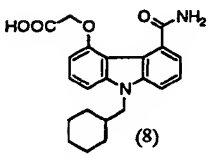
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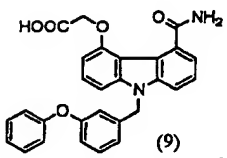
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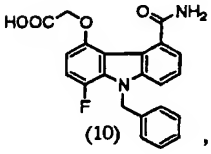
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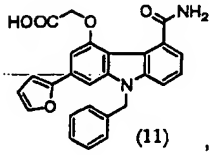
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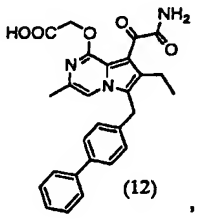
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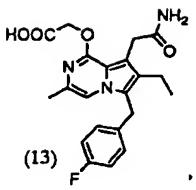
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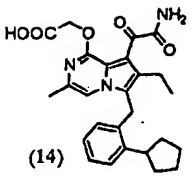
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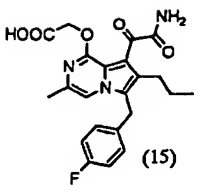
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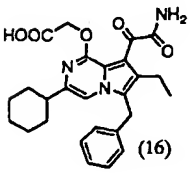
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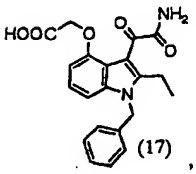
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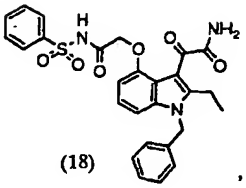
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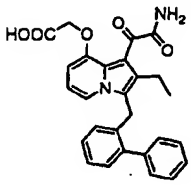

(19)

a prodrug thereof, its pharmaceutically acceptable salt, or its solvate.

XII) A composition for prevention or treatment of cancer as described in any one of I) to XI) wherein the cancer is colon cancer, lung cancer, liver cancer, gastric cancer, renal cancer, gellbladder cancer, prostatic cancer, pancreatic cancer, testis cancer, ovary cancer or cutaneous cancer.

XIII) A composition for prevention or treatment of cancer as described in any one of I) to XI) wherein the cancer is colon cancer.

XIV) Use of a type-X $sPLA_2$ inhibitor for the preparation of a medicament for the treatment of cancer.

XV) Use as described in XIV) wherein the type-X $sPLA_2$ inhibitor is the compound described in any one of II) to XI).

XVI) A method of treating a mammal, including a human, to alleviate the pathological effects of cancer, which comprises administration to said mammal of a type-X $sPLA_2$ inhibitor in a pharmaceutically effective amount.

XVII) A method of treating a mammal as described in XVI) wherein the type-X $sPLA_2$ inhibitor is the compound described in any one of II) to XI).

[0012] The present invention is illustrated in detail as follows

[0013] Type-X $sPLA_2$ inhibitors mean compounds which have an inhibitory activity against type-X $sPLA_2$ and other optional activities such as inhibitory activities against other enzymes or affinities for any receptors. Namely, the inhibitors include any compound having stronger activities against type-X $sPLA_2$ than that having no such activities in the evaluation test therefore. Especially, type-X $sPLA_2$ selective inhibitors are preferred as type-X $sPLA_2$ inhibitors of the present invention. For example, compounds whose IC_{50} values against type-X $sPLA_2$ are 1 μM or less in the experiment of Example 2 are preferred. Compounds having IC_{50} values 100 nM or less are more preferred.

[0014] A type-X $sPLA_2$ inhibitor, a compound having type-X $sPLA_2$ inhibitory activities, having one or more of chiral center(s), may exist as an optically active member. Likewise, a compound containing alkenyl or alkenylene, may be a cis- or trans-isomer. Mixtures of R- and S-isomers as well as of cis- and trans-isomers, and mixtures of R- and S-isomers containing a racemic mixture are included in the scope of the present invention. An asymmetric carbon atom may exist also in a substituent such as alkyl group. All such isomers and mixtures are included in the present invention. A specified stereoisomer can be manufactured by subjecting to stereospecific reaction well known to those skilled in

the art applying a previously separated starting material having an asymmetrical center or by preparing a mixture of stereoisomers and separating the mixture in accordance with a well-known manner.

[0015] Prodrug is a derivative of a compound with type-X $sPLA_2$ inhibitory activities, having a group which can be decomposed chemically or metabolically, and becoming pharmaceutically active by solvolysis or in vivo under a physiological condition. Although the derivative, acid derivative or basic derivative, exhibits activity, an acid derivative is more advantageous in solubility, tissue affinity, and release control in mammal organism (Bungard, H., Design of Prodrugs, pp. 7-9, 21-24. Elsevier, Amsterdam, 1985). For instance, prodrugs, including an acid derivative such as an ester which is prepared by reacting a basal acid compound with a suitable alcohol, or an amide which is prepared by reacting a basal acid compound with a suitable amine, are well known to those skilled in the art. Simple aliphatic or aromatic esters derived from acid groups contained in the compounds according to the present invention are preferable prodrugs. Particularly preferred esters as prodrugs are C1-C6 alkylester (e.g. methyl ester, ethyl ester). Double ester such as (acyloxy)alkyl ester or ((alkyloxycarbonyl)oxy)-alkyl ester type prodrugs may be optionally manufactured.

[0016] When a compound having type-X $sPLA_2$ inhibitory activities has an acidic or basic functional group, a variety of salts having a higher water solubility and more physiologically suitable properties than those of the original compound can be formed. An example of typical pharmaceutically acceptable salts includes salts with alkali metal and alkaline earth metal such as lithium, sodium, potassium, magnesium, aluminum and the like, but it is to be noted that such pharmaceutically acceptable salts are not limited thereto. A salt is easily manufactured from a free acid by either treating an acid in a solution with a base, or allowing an acid to be in contact with an ion exchange resin. Addition salts of the compounds having type-X $sPLA_2$ inhibitory activities with relatively non-toxic inorganic bases and organic bases, for example, amine cation, ammonium, and quaternary ammonium derived from nitrogenous bases having a basicity sufficient for forming a salt of the compounds of the present invention are included in the definition of "pharmaceutically acceptable salts". (e.g., S. M. Berge et al., "Pharmaceutical Salts, "J. Phar. Sci., 66, 1-19 (1977)). Furthermore, basic groups of a compound having type-X $sPLA_2$ inhibitory activities are reacted with a suitable organic or inorganic acid to form salts such as acetates, benzenesulfonates, benzoates, bicarbonates, bisulfates, bitartrate, borates, bromides, camsylates, carbonates, chlorides, clavulanates, citrates, edetates, edisylates, estolates, esylates, fluorides, fumarates, gluceptates, gluconates, glutamates, glycolylarsanilates, hexylresorcinates, hydroxynaphthoates, iodides, isothionates, lactates, lactobionates, laurates, malates, maleates, mandelates, mesylates, methylbromides, methylnitrates, methylsulfates, mucates, napsylates, nitrates, oleates, oxalates, palmitates, pantothenates, phosphates, polygalacturonates, salicylates, stearates, subacetates, succinates, tannates, tartrates, tosylates, trifluoroacetates, trifluoromethanesulfonates, valerates and the like.

[0017] The solvate includes solvates with organic solvents and/or hydrates. In case of forming a hydrate, a questioned compound may be coordinated with a suitable number of water molecules.

[0018] The term "pharmaceutically acceptable" means that carriers, diluents, or additives are compatible with other ingredients in a formulation and are not harmful for recipients.

[0019] The "cancer" means various malignant tumors originated from epithelial cells in various tissues, cells such as colon cancer, lung cancer, liver cancer, gastric cancer, renal cancer, gallbladder cancer, prostate cancer, pancreatic cancer, testis cancer, ovarian cancer, cutaneous cancer, esophagus cancer, laryngeal cancer, breast cancer or uterine cancer and exemplified. Based on the experiments, the inventor of the present invention confirmed the elevated expression of type-X $sPLA_2$ in various tumor tissues, including colon cancer, lung cancer, liver cancer, gastric cancer, renal cancer, gallbladder cancer, prostate cancer, pancreatic cancer, testis cancer, ovarian cancer or cutaneous cancer. Especially, the present invention is useful for the prevention or treatment of colon cancer, lung cancer, liver cancer, gastric cancer, renal cancer, gallbladder cancer, prostate cancer, pancreatic cancer, testis cancer, ovarian cancer or cutaneous cancer.

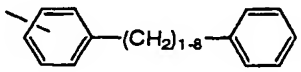
[0020] In the present specification, the term "alkyl" employed alone or in combination with other terms means a straight- or branched chain monovalent hydrocarbon group having a specified number of carbon atoms. An example of the alkyl includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decanyl, n-undecanyl, n-dodecanyl, n-tridecanyl, n-tetradecanyl, n-pentadecanyl, n-hexadecanyl, n-heptadecanyl, n-octadecanyl, n-nonadecanyl, n-eicosanyl and the like.

[0021] The term "alkenyl" employed alone or in combination with other terms in the present specification means a straight- or branched chain monovalent hydrocarbon group having a specified number of carbon atoms and at least one double bond. An example of the alkenyl includes vinyl, allyl, propenyl, crotonyl, isopentenyl, a variety of butenyl isomers and the like.

[0022] The term "alkynyl" used in the present specification means a straight or branched chain monovalent hydrocarbon group having a specified number of carbon atoms and at least one triple bond. The alkynyl may contain (a) double bond(s). An example of the alkynyl includes ethynyl, propynyl, 6-heptynyl; 7-octynyl, 8-nonynyl and the like.

[0023] The term "carbocyclic group" used in the present specification means a group derived from a saturated or unsaturated, substituted or unsubstituted 5 to 14 membered, preferably 5 to 10 membered, and more preferably 5 to 7 membered organic nucleus whose ring forming atoms (other than hydrogen atoms) are solely carbon atoms. A group

containing two to three of the carbocyclic group is also included in the above stated group. An example of typical carbocyclic groups includes cycloalkyl such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl, cycloalkenyl such as cyclobutylenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, and cyclooctenyl, phenyl, naphthyl, norbornyl, bicycloheptadienyl, indenyl, stilbenyl, terphenylyl, phenylcyclohexenyl, acenaphthyl, anthryl, biphenylyl, bibenzyl, and a phenylalkylphenyl derivative represented by the formula (II):



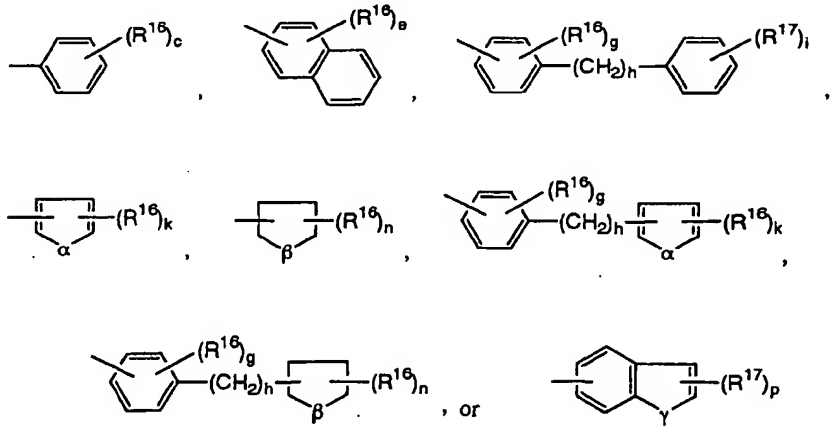
(II)

[0024] Phenyl, cycloalkyl or the like is preferred as a carbocyclic groups in the R^3 and R^4.

[0025] The term "heterocyclic group" used in the present specification means a group derived from monocyclic or polycyclic, saturated or unsaturated, substituted or unsubstituted heterocyclic nucleus having 5 to 14 ring atoms and containing 1 to 3 hetero atoms selected from the group consisting of nitrogen atom, oxygen atom, and sulfur atom. An example of the heterocyclic group includes pyridyl, pyrrolyl, furyl, benzofuryl, thienyl, benzothienyl, pyrazolyl, imidazolyl, phenylimidazolyl, triazolyl, isoxazolyl, oxazolyl, thiazolyl, thiadiazolyl, indolyl, carbazolyl, norharmanyl, azaindolyl, benzofuranyl, dibenzofuranyl, dibenzothiophenyl, indazolyl, imidazo[1,2-a]pyridinyl, benzotriazolyl, anthranilyl, 1,2-benzisoxazolyl, benzoxazolyl, benzothiazolyl, purinyl, puridinyl, dipyridinyl, phenylpyridinyl, benzylpyridinyl, pyrimidinyl, phenylpyrimidinyl, pyrazinyl, 1,3,5-triazinyl, quinolyl, phthalazinyl, quinazolinyl, quinoxalinyl, and the like.

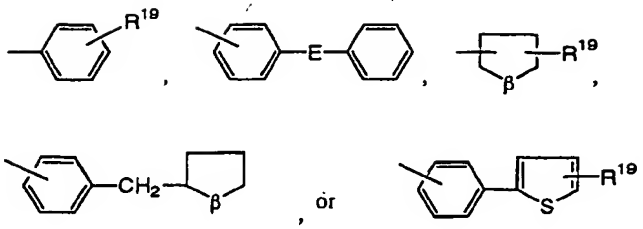
[0026] Furyl, thienyl or the like is preferred as a heterocyclic group in the R^3 and R^4.

[0027] Preferred carbocyclic and heterocyclic groups in R^5 represented by the formula:



wherein h is an integer from 0 to 2, R^{16} and R^{17} are each independently halogen, C1-C10 alkyl, C1-C10 alkyloxy, C1-C10 alkylthio, aryloxy, or C1-C10 haloalkyl, α is oxygen atom or sulfur atom, β is $-CH_2-$ or $-(CH_2)_2-$, γ is oxygen atom or sulfur atom, c, i, and p are each independently an integer from 0 to 5, e is an integer from 0 to 7, g is an integer from 0 to 4, k and n are each independently an integer from 0 to 3. When the above c, e, g, i, k, n, and/or p are 2 or more, a plural number of R^{16} or R^{17} may be different from one another. When R^{16} is a substituent on the naphthyl group, the substituent may be substituted at any arbitrary position on the naphthyl group.

[0028] A more preferable example includes a group represented by the formula:



wherein R^{19} is hydrogen atom, C1-C3 alkyl or halogen; E is a bond, $-CH_2-$, or -O-; β is $-CH_2-$ or $-(CH_2)_2-$as defined above.

[0029] The above-mentioned "carbocyclic ring" C1-C3 alkyl and the above-mentioned "heterocyclic ring" C1-C3 alkyl, or the like is preferred as a group in the R^5.

[0030] The term "non-interfering substituent" in the present specification means a group suitable for substitution of the above mentioned "carbocyclic group", "heterocyclic group", and basic skeleton. An example of the non-interfering substituents includes C1-C10 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, C7-C12 aralkyl such as benzyl and phenethyl, C7-C12 alkaryl, C3-C8 cycloalkyl, C3-C8 cycloalkenyl, phenyl, tolyl, xylyl, biphenylyl, C1-C10 alkyloxy, C1-C6 alkyloxy C1-C6 alkyl such as methyloxymethyl, ethyloxymethyl, methyloxyethyl, and ethyloxyethyl, C1-C6 alkyloxy C1-C6 alkyloxy such as methyloxymethyloxy and methyloxyethyloxy, C1-C6 alkylcarbonyl such as methylcarbonyl and ethylcarbonyl, C1-C6 alkylcarbonylamino such as methylcarbonylamino and ethylcarbonylamino, C1-C6 alkyloxyamino such as methyloxyamino and ethyloxyamino, C1-C6 alkyloxyaminocarbonyl such as methyloxyaminocarbonyl and ethyloxyaminocarbonyl, mono or di C1-C6 alkylamino such as methylamino, ethylamino, dimethylamino, and ethylmethylamino, C1-C10 alkylthio, C1-C6 alkylthiocarbonyl such as methylthiocarbonyl and ethylthiocarbonyl, C1-C6 alkylsulfinyl such as methylsulfinyl and ethylsulfinyl, C1-C6 alkylsulfonyl such as methylsulfonyl and ethylsulfonyl, C2-C6 haloalkyloxy such as 2-chloroethyloxy and 2-bromoethyloxy, C1-C6 haloalkylsulfonyl such as chloromethylsulfonyl and bromomethylsulfonyl, C1-C10 haloalkyl, C1-C6 hydroxyalkyl such as hydroxymethyl and hydroxyethyl, C1-C6 alkyloxycarbonyl such as methyloxycarbonyl and ethyloxycarbonyl, $-(CH_2)_{1-8}$-O-(C1-C6 alkyl), benzyloxy, aryloxy such as phenyloxy, arylthio such as phenylthio, $-(CONHSO_2R^{20})$ wherein R^{20} is C1-C6 alkyl or aryl, -CHO, amino, amidino, halogen, carbamyl, carboxyl, carbalkoxy, $-(CH_2)_{1-8}$-COOH such as carboxymethyl, carboxyethyl, and carboxypropyl, cyano, cyanoguanidino, guanidino, hydrazide, hydrazino, hydroxy, hydroxyamino, nitro, phosphono, $-SO_3H$, thioacetal, thiocarbonyl, C1-C6 carbonyl, a carbocyclic group, a heterocyclic group and the like. These are optionally substituted with one or more substituents selected from the group consisting of C1-C6 alkyl, C1-C6 alkyloxy, C2-C6 haloalkyloxy, C1-C6 haloalkyl, and halogen.

[0031] Preferable are halogen, C1-C6 alkyl, C1-C6 alkyloxy, C1-C6 alkylthio, and C1-C6 haloalkyl as the "non-interfering substituent" of "substituted with non-interfering substituent" in the R^3, R^4, and R^5. More preferable are halogen, C1-C3 alkyl, C1-C3 alkyloxy, C1-C3 alkylthio, and C1-C3 haloalkyl.

[0032] Preferable are C1-C6 alkyl, aralkyl, C1-C6 alkyloxy, C1-C6 alkylthio, C1-C6 hydroxyalkyl, C2-C6 haloalkyloxy, halogen, carboxy, C1-C6 alkyloxycarbonyl, aryloxy, arylthio, a carbocyclic group, and a heterocyclic group as the "non-interfering substituent" in the R^1, R^2, R^3, R^4, and R^7. More preferable are C1-C6 alkyl, aralkyl, carboxy, C1-C6 hydroxyalkyl, phenyl, and C1-C6 alkyloxycarbonyl.

[0033] The term "halogen" in the present specification means fluorine, chlorine, bromine, and iodine.

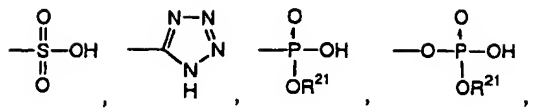
[0034] The term "cycloalkyl" in the present specification means a monovalent cyclic hydrocarbon group having a specified number of carbon atoms. An example of the cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like.

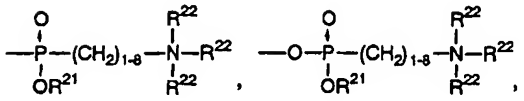
[0035] The term "cycloalkenyl" in the present specification means a monovalent cyclic hydrocarbon group having a specified number of carbon atoms and at least one double bond(s). An example of the cycloalkenyl includes 1-cyclopropenyl, 2-cyclopropenyl, 1-cyclobutenyl, 2-cyclobutenyl and the like.

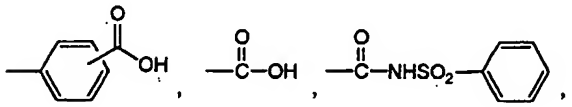
[0036] In the present specification, an example of "alkyloxy" includes methyloxy, ethyloxy, n-propyloxy, isopropyloxy, n-butyloxy, n-pentyloxy, n-hexyloxy and the like.

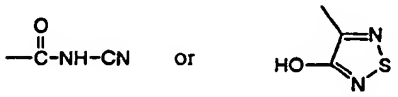
[0037] In the present specification, an example of "alkylthio" includes methylthio, ethylthio, n-propylthio, isopropylthio, n-butylthio, n-pentylthio, n-hexylthio and the like.

[0038] The term "acidic group" in the present specification means an organic group functioning as a proton donor capable of hydrogen bonding when attached to a basic skeleton through a suitable linking atom (hereinafter defined as "acid linker"). An example of the acidic group includes a group represented by the formula:



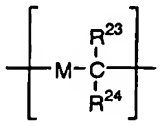




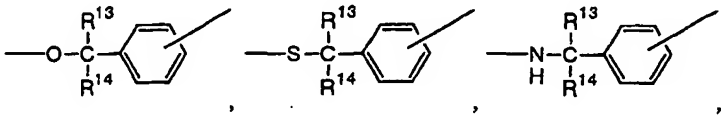


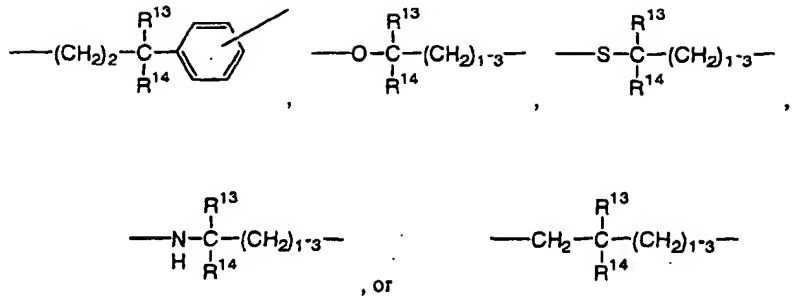
wherein R^{21} is hydrogen atom, a metal, or C1-C10 alkyl; each R^{22} is independently hydrogen atom or C1-C10 alkyl, provided that at least one of R^{21} or R^{22} is hydrogen atom in case of an acidic group having both R^{21} and R^{22}. Preferable is -COOH, $-SO_3H$, $-CONHSO_2C_6H_5$, or $P(O)(OH)_2$. More preferable is -COOH.

[0039] The term "acid linker" in the present specification means a divalent linking group represented by a symbol $-(L^1)-$, and it functions to join a basic skeleton to an "acidic group" in the general relationship. An example of it includes a group represented by the formula:



wherein M is $-CH_2-$, -O-, $-N(R^{25})-$, or -S- wherein R^{23} and R^{24} are each independently hydrogen atom, C1-C10 alkyl, aryl, aralkyl, carboxy, or halogens and a group represented by the formula:





wherein $R^{13}$and R^{14} are each independently hydrogen atom, C1-C10 alkyl, C1-C10 aralkyl, carboxy, alkyloxycarbonyl, or halogen. Preferable are -O-CH_2-, -S-CH_2-, -N(R^{25})-CH_2-, -CH_2-CH_2-, -O-CH(CH_3)-, or -O-CH((CH_2)$_2$$C_6H_5$)- wherein R^{25} is C1-C6 alkyl. More preferable is -O-CH_2- or -S-CH_2-.

[0040] In the present specification, the term "acid linker length" means the number of atoms (except for hydrogen atoms) in the shortest chain of a linking group -(L^1)- which connects a basic skeleton with the "acidic group". The presence of a carbocyclic ring in -(L^1)- counts as the number of atoms approximately equivalent to the calculated diameter of the carbocyclic ring. Thus, a benzene and cyclohexane ring in the acid linker counts as two atoms in calculating the length of -(L^1)-. A preferable length is 2 to 3.

[0041] The term "haloalkyl" in the present specification means the aforementioned "alkyl" substituted with the aforementioned "halogen" at arbitrary position(s). An example of the haloalkyl includes chloromethyl, trifluoromethyl, 2-chloromethyl, 2-bromomethyl and the like.

[0042] The term "hydroxyalkyl" in the present specification means the aforementioned "alkyl" substituted with hydroxy at arbitrary position(s). An example of the hydroxyalkyl includes hydroxymethyl, 2-hydroxyethyl, 3-hydroxypropyl and the like. In this case, hydroxymethyl is preferable.

[0043] In the present specification, the term "haloalkyl" in "haloalkyloxy" is the same as defined above. An example of it includes 2-chloroethyloxy, 2-trifluoroethyloxy, 2-chloroethyloxy and the like.

[0044] The term "aryl" in the present specification means a monocyclic or condensed cyclic aromatic hydrocarbon. An example of the aryl includes phenyl, 1-naphthyl, 2-naphthyl, anthryl and the like. Particularly, phenyl and 1-naphthyl are preferred.

[0045] The term "aralkyl" in the present specification means a group wherein the aforementioned "alkyl" is substituted with the above-mentioned "aryl". Such aryl may have a bond at any substitutable position. An example of it includes benzyl, phenethyl, phenylpropyl such as 3-phenylpropyl, naphthylmethyl such as 1-naphthylmethyl and the like.

[0046] An example of the "alkyloxycarbonyl" in the present specification includes methyloxycarbonyl, ethyloxycarbonyl, n-propyloxycarbonyl and the like.

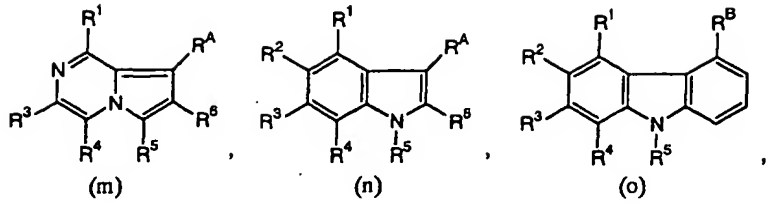
[0047] An example of the "aryloxy" in the present specification includes phenyloxy and the like.

[0048] An example of the "arylthio" in the present specification includes phenylthio and the like.

[0049] The term "halophenyl" in the present specification means phenyl substituted with the aforementioned "halogen" at one or more position(s). An example of the halophenyl includes fluorophenyl, chlorophenyl, bromophenyl, iodophenyl, difluorophenyl, dichlorophenyl, dibromophenyl, trifluorophenyl, trichlorophenyl, tribromophenyl, chlorofluorophenyl, bromochlorophenyl, and the like.

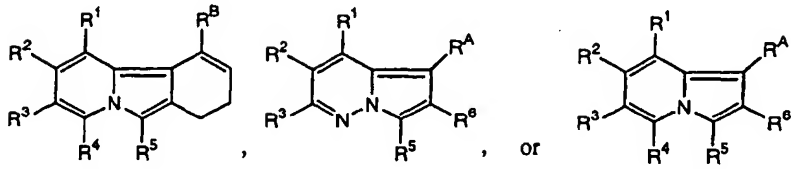
[0050] The term "cyclohexene ring " of D ring in the present specification means a cyclohexene ring having only one double bond at the condensation part with the adjacent ring.

[0051] Preferable combinations of "A ring" and "-B-" are represented by the following (m)-(r):


(m)

(n)

(o)


(p) (q) or (r)

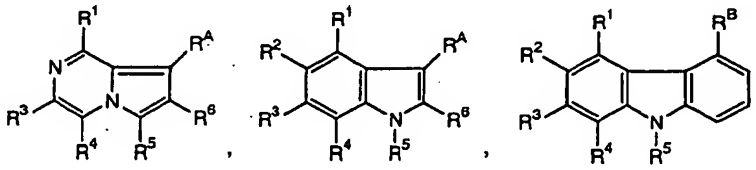
[0052] Particularly, combinations represented by (m)-(p) are preferred.

[0053] Furthermore, compounds represented by formula (1) to (19) are most preferred.

Best Mode for Carrying Out the Invention

[0054] The present invention relates to the prevention or treatment of cancer by a type-X $sPLA_2$ inhibitor. The type-X $sPLA_2$ inhibitor may be known one and selected from sPLA2 inhibitors, for example, compounds described in EP-620214 (JP Laid-Open (Tokukai) No. 95/010838, US-5578634), EP-620215 (JP Laid-Open (Tokukai) No. 95/025850, US-5684034), EP-675110 (JP Laid-Open (Tokukai) No. 95/285933, US-5654326), WO 96/03120 (JP Laid-Open No. 98/505336), WO 96/03376 (JP Laid-Open No. 98/503208, US-5641800), WO 96/03383 (JP Laid-Open No. 98/505584), WO 97/21664 (EP-779271), WO 97/21716 (EP-779273), WO 98/18464 (EP839806), WO98/24437 (EP846687), WO98/24756, WO98/24794, WO98/25609, WO99/51605, WO99/59999 and the like, or parabromophenacyl-bromide, mepacrine, manoalide, thielocin A_1 and the like.

[0055] As another type-X $sPLA_2$ inhibitor, can be used the compounds represented in PCT/JP00/07024 by the formula:



wherein R^1, R^2, R^3, and R^4 are each independently hydrogen atom, a non-interfering substituent(s) and the like, R^5 is carbocyclic groups, heterocyclic groups, R^6 is hydrogen atom, C1-C3alkyl and the like, R^A is $-COCONH_2$ and the like, R^B is $-CONH_2$, and the like.

[0056] Further, compounds identified as type-X $sPLA_2$ inhibitors by the following procedure and the like may be used in the present invention.

[0057] At first, a cell expressing human type-X $sPLA_2$ is prepared. That is, cDNA sequence encoding human type-X $sPLA_2$ (Cupillard et al., J. Biol. Chem, 1997, 272, 15745-15752) is inserted into an expression vector for mammalian cells. The obtained expression vector is transfected into the host cell and the cell stably expressing human type-X $sPLA_2$ is obtained.

[0058] Next, the above-mentioned transfected cell is cultured in medium and its culture supernatant is used for the

measurement of each enzyme activity. In order to identify and evaluate an inhibitor of type-X $sPLA_2$, the following chromogenic assay is utilized. A general explanation for this assay is described in "Analysis of Human Synovial Fluid Phospholipase A_2 on Short Chain Phosphatidylcholine-Mixed Micelles: Development of a Spectrophotometric Assay Suitable for a Micortiterplate Reader" (Analytical Biochemistry, 204, pp 190-197, 1992 by Laure. J. Reynolds. Lori L. Hughes and Edward A. Dennis.

[0059] Several kinds of the compounds represented by the formula (I) can be synthesized in accordance with the methods described in PCT/JP00/07024, EP-620214 (JP Laid-Open (Tokukai) No. 95/010838, US-5578634), EP-620215 (JP Laid-Open (Tokukai) No. 95/025850, US-5684034), EP-675110 (JP Laid-Open (Tokukai) No. 95/285933, US-5654326), WO 96/03120 (JP Laid-Open No. 98/505336), WO 96/03383 (JP Laid-Open No. 98/505584), WO 98/18464 (EP839806), WO99/51605, WO99/59999 and the like.

[0060] The composition for treatment or prevention of cancer in the present invention may be administered to a patient through a variety of routes including oral, aerosol, rectal, percutaneous, subcutaneous, intravenous, intramuscular, and nasal routes. A formulation according to the present invention may be manufactured by combining (for example, admixing) a curatively effective amount of a compound of the present invention with a pharmaceutically acceptable carrier or diluent. The formulation of the present invention may be manufactured with the use of well-known and easily available ingredients in accordance with a known method.

[0061] In case of manufacturing a composition of the present invention, active ingredients are admixed or diluted with a carrier, or they are contained in a carrier in the form of capsule, sacheier, paper, or another container. In case of functioning a carrier as a diluent, the carrier is a solid, semi-solid, or liquid material which functions as a medium. Accordingly, a formulation according to the present invention may be produced in the form of tablet, pill, powder medicine, intraoral medicine, elixir agent, suspending agent, emulsifier, dissolving agent, syrup agent, aerosol agent (solid in liquid medium), and ointment. Such a formulation may contain up to 10% of an active compound. It is preferred to formulate a compound having activities for the treatment or prevention of cancer prior to administration.

[0062] Any suitable carrier well known to those skilled in the art may be used for the formulation. In such formulation, a carrier is in the form of solid, liquid, or a mixture thereof. For instance, a compound having type-X $sPLA_2$ inhibitory activities is dissolved into 4% dextrose/0.5% sodium citrate aqueous solution so as to be 2 mg/mL concentration for intravenous injection. Solid formulation includes powder, tablet, and capsule. Solid carrier consists of one or more of material(s) for serving also as fragrant, lubricant, dissolving agent, suspension, binder, tablet disintegrator, capsule. A tablet for oral administration contains a suitable excipient such as calcium carbonate, sodium carbonate, lactose, calcium phosphate and the like together with a disintegrator such as corn starch, alginic acid and the like and/or a binder such as gelatin, acacia and the like, and a lubricant such as magnesium stearate, stearic acid, talc and the like.

[0063] In a powder medicine, a carrier is a finely pulverized solid which is blended with finely pulverized active ingredients. In a tablet, active ingredients are admixed with a carrier having required binding power in a suitable ratio, and it is solidified in a desired shape and size. Powder medicine and tablet contain about 1 to about 99% by weight of the active ingredients being novel compounds according to the present invention. An example of suitable solid carriers includes magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth gum, methyl cellulose, sodium carboxymethylcellulose, low-melting wax, and cocoa butter.

[0064] An axenic liquid formulation contains suspending agent, emulsifier, syrup agent, and elixir agent. Active ingredients may be dissolved or suspended into a pharmaceutically acceptable carrier such as sterile water, a sterile organic solvent, a mixture thereof and the like. Active ingredients may be dissolved frequently into a suitable organic solvent such as propylene glycol aqueous solution. When finely pulverized active ingredients are dispersed into aqueous starch, sodium carboxymethylcellulose solution, or suitable oil, the other compositions can be prepared.

[0065] The dosage varies with the conditions of the disease, administration route, age and body weight of patient. In the case of intravenous administration, the dosage can generally be between 0.01 to 10 mg/kg/h for adult, preferably 0.1 to 1 mg/kg/h.

Example

Example 1 Preparation of cells expressing human type-X $sPLA_2$ and their culture supernatants

[0066] cDNA sequence encoding human type-X $sPLA_2$ (Cupillard et al., J. Biol. Chem, 1997, 272, 15745-15752) was inserted into the downstream region of the promoter of pSVL SV40 Late Promoter Expression Vector (Amersham Pharmacia Biotech Inc.) that is an expression vector for mammalian cells. The obtained expression vector was transfected into the host CHO cells with a LipofectAMINE reagent (Gibco BRL Inc.) according to the manufacture's instruction to obtain the CHO cells stably expressing human type-X $sPLA_2$. The transfected cell was cultured in α-MEM medium containing 10% fetal calf serum for 3 days and its culture supernatant was used for the measurement of each enzyme activity.

Example 2 Inhibition test

[0067] In order to identify and evaluate an inhibitor of type-X $sPLA_2$, the following chromogenic assay is utilized. This assay has been applied for high volume screening using a 96-well microtiterplate. A general explanation for this assay is described in "Analysis of Human Synovial Fluid Phospholipase A_2 on Short Chain Phosphatidylcholine-Mixed Micelles: Development of a Spectrophotometric Assay Suitable for a Micortiterplate Reader" (Analytical Biochemistry, 204, pp 190-197, 1992 by Laure. J. Reynolds. Lori L. Hughes and Edward A. Dennis.

[0068] Test compounds (or solvent blank) were added according to the alignment of plates that had been previously set. Human type-X $sPLA_2$ was incubated (30 min at 40 °C (15 µl/well)) with diheptanoylthio PC (1 mM) in the presence of Triton X-100 (0.3 mM) and 5,5'-dithiobis(2-nitrobenzoic acid) (125 µM) in Tris-HCl buffer (25 mM, pH 7.5) containing $CaCl_2$ (10 mM), KCl (100 mM), and bovine serum albumin (1.0 mg/mL). The changes in the absorbance at 405 nm were measured and the inhibition activities were calculated.

[0069] The IC_{50} value was determined by plotting the log concentration of the above-mentioned compounds (1)-(19) with respect to their inhibition values within 10% to 90% inhibitory range.

[0070] Results of the type-X $sPLA_2$ inhibition test is shown in the following Table 1.

Table 1

Compound No.	IC_{50}(nM)	Compound No.	IC_{50}(nM)
1	10	11	10
2	10	12	16
3	5	13	19
4	27	14	9
5	12	15	17
6	17	16	7
7	5	17	12
8	3	18	16
9	13	19	26
10	12		

Example 3 Immunohistochemical analysis in human colon cancer tissues with anti-type-X $sPLA_2$ antibody

[0071] In this experiment, anti-type-X $sPLA_2$ antibody that was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human colon cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 µg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 µg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 µg/mL) for 2 hr before the addition to the slides.

[0072] Consequently, positive signals representative for type-X $sPLA_2$ expression were not obviously detected in normal colon, but strongly detected in the tumor cells in human colon adenocarcinoma tissues. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human colorectal cancer tissues.

Example 4 Immunohistochemical analysis in human lung cancer tissues with anti-type-X $sPLA_2$ antibody

[0073] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human lung cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0074] Consequently, positive signals representative for type-X $sPLA_2$ expression were weakly observed in type II pneumocytes in normal human lung. In contrast, the positive signals were strongly detected in tumor cells in the lung tissues prepared from patients of lung cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human lung cancer tissues.

Example 5 Immunohistochemical analysis in human liver cancer tissues with anti-type-X $sPLA_2$ antibody

[0075] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human liver cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0076] Consequently, positive signals representative for type-X $sPLA_2$ expression were weakly observed in hepatic lobule and Kupffer's satellate cells in normal human liver. In contrast, the positive signals were strongly detected in tumor cells in the liver tissues prepared from patients of liver cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human liver cancer tissues.

Example 6 Immunohistochemical analysis in human gastric cancer tissues with anti-type-X $sPLA_2$ antibody

[0077] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human gastric cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in

the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60μg/mL) for 2 hr before the addition to the slides.

[0078] Consequently, positive signals representative for type-X $sPLA_2$ expression were not obviously detected in normal gaster, but strongly detected in tumor cells in the gastric tissues prepared from patients of gastric cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human gastric cancer tissues.

Example 7 Immunohistochemical analysis in human renal cancer tissues with anti-type-X $sPLA_2$ antibody.

[0079] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human renal cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0080] Consequently, positive signals representative for type-X $sPLA_2$ expression were weakly observed in glomerular mesangial cells in normal kidney. In contrast, the positive signals were strongly detected in tumor cells in the kidney prepared from patients of renal cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human renal cancer tissues.

Example 8 Immunohistochemical analysis in human gallbladder cancer tissues with anti-type-X $sPLA_2$ antibody

[0081] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human gallbladder cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0082] Consequently, positive signals representative for type-X $sPLA_2$ expression were not obviously detected in normal gallbladder tissues, but strongly detected in tumor cells in the gallbladder tissues prepared from patients of gallbladder cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human gallbladder cancer tissues.

Example 9 Immunohistochemical analysis in human prostate cancer tissues with anti-type-X $sPLA_2$ antibody

[0083] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human prostate cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0084] Consequently, positive signals representative for type-X $sPLA_2$ expression were not obviously detected in normal prostates, but strongly detected in tumor cells in the prostate tissues prepared from patients of prostate cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human prostate cancer tissues.

Example 10 Immunohistochemical analysis in human pancreatic cancer tissues with anti-type-X $sPLA_2$ antibody

[0085] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human pancreatic cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 0.4% hematoxylin solution. Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0086] Consequently, positive signals representative for type-X $sPLA_2$ expression were not obviously detected in normal pancreas, but strongly detected in tumor cells in the pancreas prepared from patients of pancreatic cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human pancreatic cancer tissues.

Example 11 Immunohistochemical analysis in human testis cancer tissues with anti-type-X $sPLA_2$ antibody

[0087] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human testis cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in

the tissue preparations. In addition, the nuclei were counterstained with 1% methyl green dye in 0.1 mol/L sodium acetate (pH 4.0). Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0088] Consequently, positive signals representative for type-X $sPLA_2$ expression were weakly observed in some parts of the seminiferous tubules of normal testis. In contrast, the positive signals were strongly detected in malignant cells present in the seminiferous tubules and/or the epithelium of seminal vesicles in the testis prepared from patients of testis cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human testis cancer tissues.

Example 12 Immunohistochemical analysis in human ovarian cancer tissues with anti-type-X $sPLA_2$ antibody

[0089] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human ovarian cancer tissues and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 1% methyl green dye in 0.1 mol/L sodium acetate (pH 4.0). Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0090] Consequently, positive signals representative for type-X $sPLA_2$ expression were not obviously detected in normal ovary, but strongly detected in the malignant cells present in the epithelium of ovarian follicles and/or oviducts in the ovary prepared from patients of ovarian cancer. Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein was greatly elevated in human ovarian cancer tissues.

Example 13 Immunohistochemical analysis in human cutaneous cancer tissues with anti-type-X $sPLA_2$ antibody

[0091] In this experiment, anti-type-X $sPLA_2$ antibody which was described in "The Journal of Biological Chemistry Vol. 274, No. 48, pp.34203-34211 1999" was used. Paraffin embedded preparations of human cutaneous cancer tissues (malignant melanoma) and corresponding normal tissues were purchased from Biochain Inc. (San Leandro, CA). The tissue sections in the slides were dewaxed, treated in methanol containing 0.3% H_2O_2 for 30 min to remove the endogenous peroxidase activity and incubated with 5% normal goat serum for 20 min. The slides were then incubated with anti-type-X $sPLA_2$ antibody (6 μg/mL) in PBS containing 0.1% bovine serum albumin for 14 hr at 4°C. After washing with PBS, they were incubated with biotin-conjugated goat anti-rabbit IgG antibody for 30 min followed by treatment with peroxidase labelled avidin-biotin complex reagent (Vector Laboratories). After washing, the samples were processed with 200 μg/mL diaminobenzidine hydrochloride substrate dissolved in 50 mmol/L Tris-HCl (pH 7.6) containing 0.006% H_2O_2 for 10 min resulting in the appearance of color dependent on the peroxidase activity to visualize the type-X $sPLA_2$ expression in the tissue preparations. In addition, the nuclei were counterstained with 1% methyl green dye in 0.1 mol/L sodium acetate (pH 4.0). Positive signals representative for type-X $sPLA_2$ expression was visualized as a dark-brownish color of diaminobenzidine deposit. The neutralization of type-X $sPLA_2$ specific signals was conducted by incubating anti-type-X $sPLA_2$ antibody with purified type-X $sPLA_2$ protein (60 μg/mL) for 2 hr before the addition to the slides.

[0092] Consequently, positive signals representative for type-X $sPLA_2$ expression were weakly observed in the melanocytes of stratum spinosum and/or stratum basale in skin epidermis in normal human cutis. In contrast, the positive signals were strongly detected in hypertrophied melanocytes in the cutis prepared from patients of cutaneous cancer (malignant melanoma). Since the addition of type-X $sPLA_2$ protein resulted in abolishment of the signals, they were verified as the specific signals for type-X $sPLA_2$. In addition, there was no positive signal when IgG prepared from non-immunized rabbit was used. Taken together, these findings suggest that the expression of type-X $sPLA_2$ protein

was greatly elevated in human cutaneous cancer (malignant melanoma) tissues.

Example 14 Effects of $sPLA_2$ inhibitors on human type-X $sPLA_2$-induced oleic acid release in human colon carcinoma cell lines, HT-29 cells

[0093] Compound (1) and compound (19) was used as test compounds.

[0094] Human colon carcinoma cell lines, HT-29 cells (obtained from ATCC) were cultured in DMEM supplemented with 10% fetal calf serum. The cells were washed by phosphate-buffered saline (PBS), detached from culture plates by treatment with trypsin/EDTA solution and further washed by PBS. The resulting cells were resuspended in Hanks' buffered saline containing 0.1% bovine serum albumin (BSA) at a density of 12.5×10^6 cells/mL. Aliquots of the cell suspension (0.4 mL) were transferred into polypropylene tubes and test compounds dissolved in DMSO solution (final concentration; 10 μM) were added. After preincubation for 10 min at 37°C, 100 nM purified human type-X $sPLA_2$ enzyme (Hanasaki *et al.* J. Biol. Chem. (1999) 274, 34203-34211) was added (final volume of 0.5 mL). After incubation for 30 min at 37°C, the reaction was stopped by the addition of 2 mL Dole's reagent (heptane : 2-propanol : 1 M H_2SO_4 = 10 : 40 : 1, v/v/v). According to the method of Tojo *et al.* (J. lipid Res. (1993) 34, 837-844), the released fatty acids were extracted, labeled with 9-anthryldiazomethane (Funakoshi Co.), and the oleic acid was quantified by reverse-phase high performance chromatography (LiChroCART 125-4 Superspher 100 RP-18 column (Merck). From each data, the value in the absence of type-X $sPLA_2$ was subtracted. The amount of released oleic acid in the presence of each test compound was expressed as the percentage of the increased content by the addition of type-X $sPLA_2$ enzyme. As shown in Table 2, each test compound significantly inhibited the type-X $sPLA_2$ -induced oleic acid release.

Example 15 Effects of $sPLA_2$ inhibitors on type-X $sPLA_2$-induced PGE_2 production in human colon carcinoma cell lines, HT-29 cells

[0095] HT-29 cells were seeded into 24-well plates at a density of 2.5×10^5 cells/well. After incubation for 24 h, the cells were washed with PBS and incubated with 30 ng/mL of Tumor necrosis factor-α (R&D Systems, Inc.) in DMEM medium supplemented with 10% fetal bovine serum for 18 h. After washing with PBS, the cells were preincubated with or without test compounds (at a final concentration of 10 μM ; dissolved in DMSO) in Hanks' buffered saline containing 0.1% BSA for 10 min at 37°C, and then stimulated with 100 nM purified human type-X $sPLA_2$ enzyme in a final volume of 0.5 mL. After incubation for 3 h at 37°C, the culture supernatant was collected following the centrifugation for the removal of floating cells, and its PGE_2 content was quantified with an enzyme-immunoassay kit (Cayman Chemicals Co.). From each data, the value in the absence of type-X $sPLA_2$ was subtracted. The amount of PGE_2 in the presence of each test compound was expressed as the percentage of the PGE_2 content produced by the addition of type-X $sPLA_2$ enzyme. As shown in Table 2, each test compound significantly blocked the type-X $sPLA_2$-induced PGE_2 production.

[0096] Potential involvement of PGE_2 in the progression of tumors has been described in Cancer Research 59, 5093-5096, 1999. Since the compounds in the present invention significantly block the type-X $sPLA_2$-induced PGE_2 production, they can be applied as antitumor agents.

Table 2

Compound (10 μM)	Oleic acid release	PGE_2 production
No treatment group	100±2.8	100±22.7
(1)	-3.0±3.9**	-4.1±12.6**
(19)	1.8±4.6**	42.1±9.7**
Student's t-test (*P < 0.05, **P < 0.01)		

Formulation Example

[0097] It is to be noted that the following Formulation Examples 1 to 8 are mere illustration, but not intended to limit the scope of the invention. The term "active ingredient" means the compounds having an inhibitory activity against type-X PLA_2, the prodrugs thereof, their pharmaceutical acceptable salts, or their hydrate.

Formulation Example 1

[0098] Hard gelatin capsules are prepared using of the following ingredients:

	Dose (mg/capsule)
Active ingredient	250
Starch, dried	200
Magnesium stearate	10
Total	460 mg

Formulation Example 2

[0099] A tablet is prepared using of the following ingredients:

	Dose (mg/tablet)
Active ingredient	250
Cellulose, microcrystals	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665 mg

[0100] The components are blended and compressed to form tablets each weighing 665 mg.

Formulation Example 3

[0101] An aerosol solution is prepared containing the following components:

	Weight
Active ingredient	0.25
Ethanol	25.75
Propellant 22 (chlorodifluoromethane)	74.00
Total	100.00

[0102] The active compound is mixed with ethanol and the admixture added to a portion of the propellant 22, cooled to -30 °C and transferred to filling device. The required amount is then fed to stainless steel container and diluted with the reminder of the propellant. The valve units are then fitted to the container.

Formulation Example 4

[0103] Tablets, each containing 60 mg of active ingredient, are made as follows.

Active ingredient	60 mg
Starch	45 mg
Microcrystals cellulose	35 mg
Polyvinylpyrrolidone (as 10% solution in water)	4 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1 mg
Total	150 mg

[0104] The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve, and the mixed thoroughly. The aqueous solution containing polyvinylpyrrolidone is mixed with the resultant powder, and the admixture then is passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through

No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation Example 5

[0105] Capsules, each containing 80 mg of active ingredient, are made as follows:

Active ingredient	80 mg
Starch	59 mg
Microcrystals cellulose	59 mg
Magnesium stearate	2 mg
Total	200 mg

[0106] The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation Example 6

[0107] Suppository, each containing 225 mg of active ingredient, are made as follows:

Active ingredient	225 mg
Saturated fatty acid glycerides	2000 mg
Total	2225 mg

[0108] The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2g capacity and allowed to cool.

Formulation Example 7

[0109] Suspensions, each containing 50 mg of active ingredient per 5 mL dose, are made as follows:

Active ingredient	50 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mL
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to total	5 mL

[0110] The active ingredient is passed through a No. 45 U.S. sieve, and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution and flavor are diluted with a portion of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

[0111] An intravenous formulation may be prepared as follows:

Active ingredient	100 mg
Saturated fatty acid glycerides	1000 mL

[0112] The solution of the above ingredients generally is administered intravenously to a subject at a rate of 1 mL per minute.

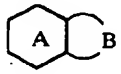
Industrial Applicability

[0113] It is provided that type X inhibitors are useful in preventing or treating cancer.

Claims

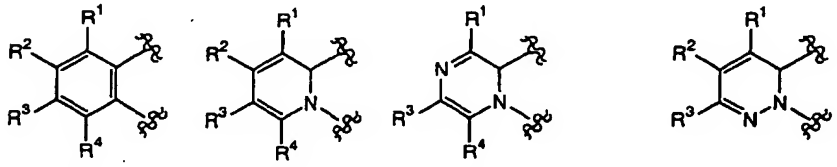
1. A composition for prevention or treatment of cancer which contains a type-X $sPLA_2$ inhibitor as an active ingredient.

2. A composition for prevention or treatment of cancer which contains as an active ingredient a compound represented by the formula (I):



(I)

wherein Ring A is represented by the formula (a) to (d):

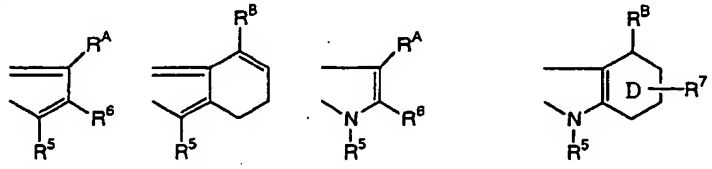


(a) , (b) , (c) , or (d)

wherein R^1 and R^2 are each independently hydrogen atom, non-interfering substituent, or -(L^1)-(acidic group) wherein L^1 is an acid linker having an acid linker length of 1 to 5, provided that one of the R^1 and R^2 is -(L^1)-(acidic group);

R^3 and R^4 are each independently hydrogen atom, non-interfering substituent, carbocyclic group, carbocyclic group substituted with a non-interfering substituent(s), heterocyclic group, or heterocyclic group substituted by a non-interfering substituent(s); and

-B- is represented by the formula (e) to (h):



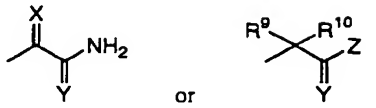
(e) , (f) , (g) , or (h)

wherein R^5 is (j) C1 to C20 alkyl, C2 to C20 alkenyl, C2 to C20 alkynyl, carbocyclic group, or heterocyclic group, (k) the group represented by (j) each substituted independently with at least one group selected from non-interfering substituents, or -(L^2)-R^8 wherein L^2 is a divalent linking group of 1 to 18 atom(s) selected from hydrogen atom(s), nitrogen atom(s), carbon atom(s), oxygen atom(s), and sulfur atom(s), and

R^8 is a group selected from the groups (j) and (k);

R^6 is hydrogen atom, halogen, C1 to C3 alkyl, C3 to C4 cycloalkyl, C3 to C4 cycloalkenyl, C1 to C3 alkyloxy, or C1 to C3 alkylthio;

R^7 is hydrogen atom or non-interfering substituent;
R^A is represented by the formula:

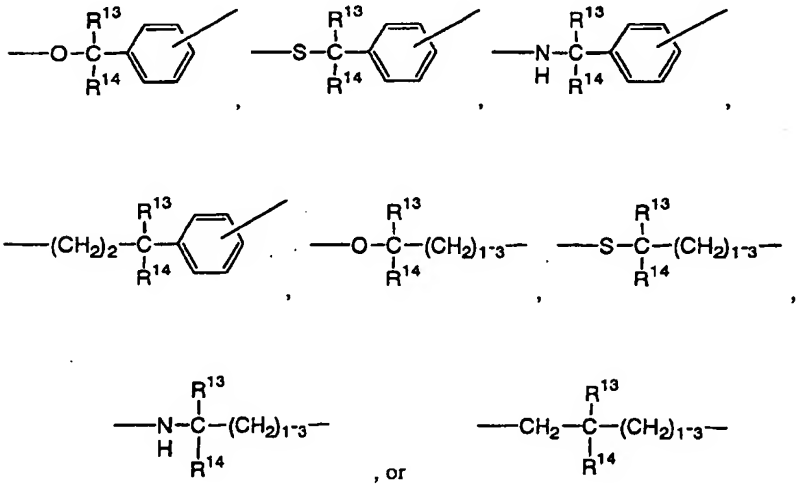


wherein R^9 and R^{10} are each independently hydrogen atom, C1 to C3 alkyl, or halogen;

X and Y are each independently oxygen atom or sulfur atom; and
Z is $-NH_2$ or $-NHNH_2$;
R^B is $-CONH_2$ or $-CONHNH_2$; and,
Ring D is cyclohexene ring or benzene ring;

provided that Ring A is (b), (c), or (d) when -B- is (e) or (f), a prodrug thereof, its pharmaceutically acceptable salt, or its solvate.

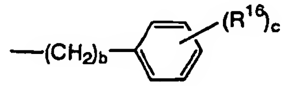
3. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^1 is hydrogen atom or $-(L^3)-R^{11}$ wherein L^3 is $-OCH_2-$, $-SCH_2-$, $-NH-CH_2-$, $-CH_2-CH_2-$, $-O-CH(CH_3)-$, or $-O-CH(CH_2CH_2C_6H_5)-$; R^{11} is $-COOH$, $-CONHSO_2C_6H_5$, $-SO_3H$, or $-P(O)(OH)_2$; and
R^2 is hydrogen atom or $-(L^4)-R^{12}$ wherein L^4 is represented by the formula:



wherein R^{13} and R^{14} are each independently hydrogen atom, C1 to C10 alkyl, C1 to C10 aralkyl, carboxy, alkyloxycarbonyl, or halogen; R^{12} is $-COOH$, $-SO_3H$, or $-P(O)(OH)_2$, provided R^1 and R^2 are not hydrogen atom at the same time.

4. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^3 is hydrogen atom, C1 to C6 alkyl, C3 to C6 cycloalkyl, aryl, or a heterocyclic group and R^4 is hydrogen atom or halogen.

5. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^5 is $-(CH_2)_{1-6}-R^{15}$ wherein R^{15} is represented by the formula: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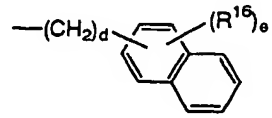
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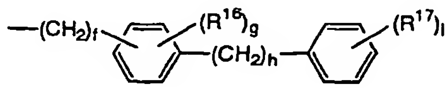
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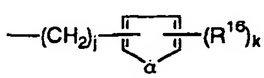
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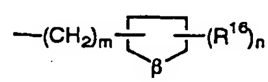

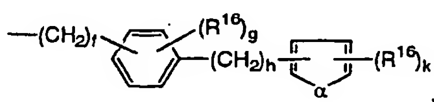
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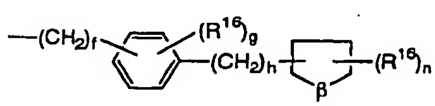
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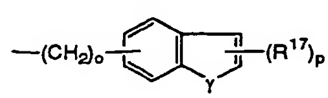
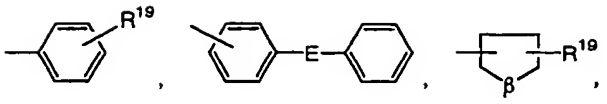
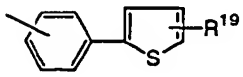


wherein b, d, f, h, j, m, and o are independently an integer from 0 to 2; R^{16} and R^{17} are each independently halogen, C1 to C10 alkyl, C1 to C10 alkyloxy, C1 to C10 alkylthio, aryloxy, or C1 to C10 haloalkyl; α is oxygen atom or sulfur atom; β is $-CH_2-$ or $-(CH_2)_2-$; γ is oxygen atom or sulfur atom; c, i, and p are independently an integer from 0 to 5; e is an integer from 0 to 7; g is an integer from 0 to 4; k and n are each independently an integer from 0 to 3.

6. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 5 as an active ingredient, wherein R^5 is $-CH_2-R^{18}$ wherein R^{18} is represented by the formula:



or



wherein β is $-CH_2-$ or $-(CH_2)_2-$; R^{19} is hydrogen atom, C1 to C3 alkyl, or halogen; E is a bond, $-CH_2-$ or -O-.

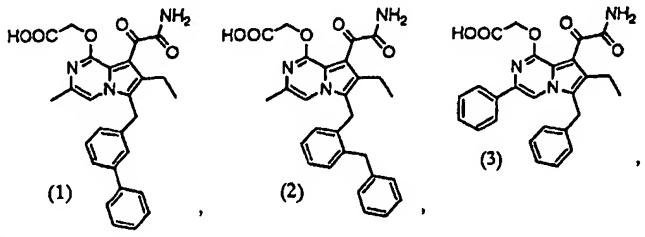
7. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^1 is $-OCH_2COOH$.

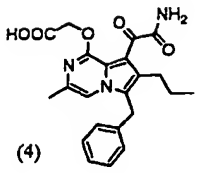
8. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^2 is hydrogen atom.

9. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^6 is C1 to C3 alkyl.

10. A composition for prevention or treatment of cancer which contains a compound, a prodrug thereof, its pharmaceutically acceptable salt, or its solvate as claimed in claim 2 as an active ingredient, wherein R^A is $-CH_2CONH_2$ or $-COCONH_2$.

11. A composition for prevention or treatment of cancer which contains a compound as an active ingredient represented by the formula:

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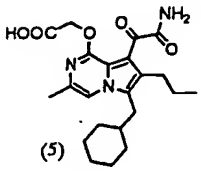
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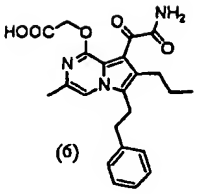
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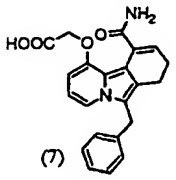
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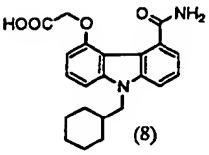
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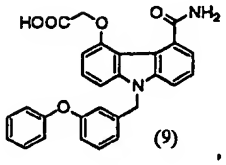
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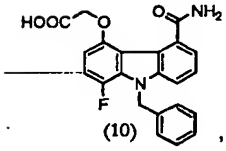
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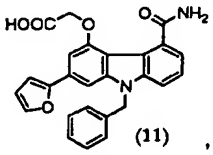
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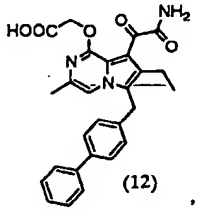
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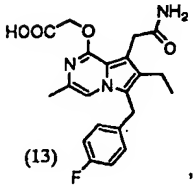
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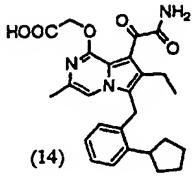
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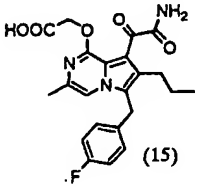
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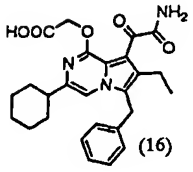
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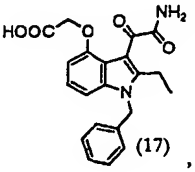
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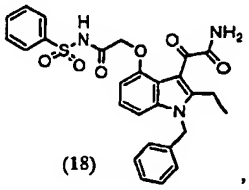
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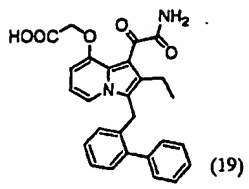
or  (19)

a prodrug thereof, its pharmaceutically acceptable salt, or its solvate.

12. A composition for prevention or treatment of cancer as claimed in any one of claims 1 to 11 wherein the cancer is colon cancer, lung cancer, liver cancer, gastric cancer, renal cancer, gellbladder cancer, prostatic cancer, pancreatic cancer, testis cancer, ovary cancer or cutaneous cancer.

13. A composition for prevention or treatment of cancer as claimed in any one of claims 1 to 11 wherein the cancer is colon cancer.

14. Use of a type-X $sPLA_2$ inhibitor for the preparation of a medicament for the treatment of cancer.

15. Use as claimed in claim 14 wherein the type-X $sPLA_2$ inhibitor is the compound claimed in any one of claims 2 to 11.

16. A method of treating a mammal, including a human, to alleviate the pathological effects of cancer, which comprises administration to said mammal of a type-X $sPLA_2$ inhibitor in a pharmaceutically effective amount.

17. A method of treating a mammal as claimed in claim 16 wherein the type-X $sPLA_2$ inhibitor is the compound claimed in any one of claims 2 to 11.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/05480

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K45/00, A61K31/4985, 31/437, 31/5025, 31/403, 31/404, A61P35/00 // C07D487/04, 471/04, 209/86, 405/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K45/00, A61K31/4985, 31/437, 31/5025, 31/403, 31/404

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1940-1992 Toroku Jitsuyo Shinan Koho 1994-1996
Kokai Jitsuyo Shinan Koho 1971-1992 Jitsuyo Shinan Toroku Koho 1996-2001

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS (STN), BIOSIS (STN), MEDLINE (STN), EMBASE (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MURAKAMI M. et al., "Different functional aspects of the group II subfamily (Types IIA and V) and type X secretory phospholipase A(2)s in regulating arachidonic acid release and prostaglandin generation. Implications of cyclooxygenase-2 induction and phospholipid scramblase-mediated cellular membrane perturbation", Journal of Biological Chemistry, 29 October, 1999, Vol.274, No.44, pages 31435 to 31444; the whole document	1,12-14
Y	EP 937722 A1 (Pfizer Inc.), 25 August, 1999 (25.08.99), page 2, lines 12 to 33 & JP 11-263788 A	1-15
Y	WATANABE K. et al., "Role of the Prostaglandin E Receptor Subtype EP1 in Colon Carcinogenesis", Cancer Research, (1999), Vol.59, pages 5093 to 5096; the whole document, especially, abstract	1-15

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 September, 2001 (07.09.01)	18 September, 2001 (18.09.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16,17
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 16 and 17 pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iV) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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Claims 1 and 12 to 14 relate to inventions of the use of active ingredients which are defined by the desired property as "type X $sPLA_2$ inhibitors". Although claim 1 involves in its scope any compounds having this property, it is recognized that only small part of the claimed compounds are exclusively supported in the description under the provision of Article 6 of the PCT and disclosed therein under the provision of Article 5 of the PCT.

Even though the common technical knowledge at the point of the application is taken into consideration, it cannot be recognized the scope of compounds having the property as "type X $sPLA_2$ inhibitors" could be specified. Thus, claims 1 and 12 to 14 also fail to satisfy the requirement of clearness as defined in Article 6 of the PCT.

Moreover, claims 2 to 10, 13 and 15 involve an extremely large number of compounds. However, it is recognized that only small part of the claimed compounds are exclusively supported in the description under the provision of Article 6 of the PCT and disclosed therein under the provision of Article 5 of the PCT.

Therefore, the search has been practiced on the relationship between the type X $sPLA_2$ inhibitory activity and cancer and preventives or remedies for cancer containing the compounds specified in claim 11 as the active ingredient.

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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 952149 A2 (Eli Lilly and Co.), 27 October, 1999 (27.10.99), the whole document & JP 11-322713 A & NO 9901821 A & AU 9923817 A & CN 1240210 A & BR 9902365 A	2-13,15
Y	EP 675110 A1 (Lilly, Eli, and Co.), 04 August, 1995 (04.08.95), the whole document & CA 2146097 A & AU 9516217 A1 & JP 7-285933 A & CN 1114310 A & BR 9501404 A & HU 72048 A & US 5654326 A	2-13,15
PX	MORIOKA Y. et al., "Potential role of group X secretory phospholipase A2 in cyclooxygenase-2-dependent PGE2 formation during colon tumorigenesis", FEBS Lett., 29 December, 2000, Vol.487, No.2, pages 262 to 266	1,12-14
PA	WO 01/26653 A1 (Shionogi & Co., Ltd.), 19 April, 2001 (19.04.01), (Family: none)	1-15

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